

Northumbria Research Link

Citation: Gerber, Ruan, Howatson, Glyn, Greenfield, Richard, Wagenaar, Ina and Smit, Nico (2017) Physiological response to angling of Africa's premier freshwater angling species, the tigerfish *Hydrocynus vittatus*. *African Zoology*, 52 (2). pp. 91-98. ISSN 1562-7020

Published by: Taylor & Francis

URL: <https://doi.org/10.1080/15627020.2017.1300069>
<<https://doi.org/10.1080/15627020.2017.1300069>>

This version was downloaded from Northumbria Research Link:
<http://nrl.northumbria.ac.uk/id/eprint/30752/>

Northumbria University has developed Northumbria Research Link (NRL) to enable users to access the University's research output. Copyright © and moral rights for items on NRL are retained by the individual author(s) and/or other copyright owners. Single copies of full items can be reproduced, displayed or performed, and given to third parties in any format or medium for personal research or study, educational, or not-for-profit purposes without prior permission or charge, provided the authors, title and full bibliographic details are given, as well as a hyperlink and/or URL to the original metadata page. The content must not be changed in any way. Full items must not be sold commercially in any format or medium without formal permission of the copyright holder. The full policy is available online: <http://nrl.northumbria.ac.uk/policies.html>

This document may differ from the final, published version of the research and has been made available online in accordance with publisher policies. To read and/or cite from the published version of the research, please visit the publisher's website (a subscription may be required.)

1 **Physiological response to angling of Africa's premier freshwater angling**
2 **species, the tigerfish, *Hydrocynus vittatus***

3
4 Ruan Gerber^{1,2*}, Glyn Howatson^{1,3}, Richard Greenfield², Ina Wagenaar² and Nico
5 Smit¹

6
7 ¹Water Research Group, Unit for Environmental Sciences and Management, North-
8 West University, Private Bag X6001, Potchefstroom 2520, South Africa

9 ²Centre for Aquatic Research, Department of Zoology, University of Johannesburg, PO
10 Box 524, Auckland Park, Johannesburg, 2006, South Africa

11 ³Faculty of Health and Life Sciences, Northumbria University, Newcastle upon Tyne,
12 UK

13
14 *gerberri@gmail.com

15
16
17 **Abstract**

18 Angling fraternities widely promote catch-and-release (C&R) as a fisheries
19 management tool. This study aimed to determine the physiological response of Africa's
20 premier freshwater angling species, the tigerfish, *Hydrocynus vittatus*, to (C&R) angling
21 in the Okavango Delta, Botswana. Standard angling techniques were used to collect
22 fish, where after fish were anaesthetised in clove oil, blood drawn from the caudal vein
23 and general biometric data noted, then revived and released. Blood plasma was
24 analysed for glucose, cortisol and lactate concentrations to assess the effects of
25 angling duration, fish size and fish condition. Larger fish were angled for a longer
26 duration. Plasma glucose concentrations decreased with greater lactate
27 concentrations, an indication of the aerobic and anaerobic work done during capture.
28 Few individuals showed increased plasma cortisol concentrations. In extended-
29 capture fish (angled for >1 min), lactate concentrations increased significantly above
30 values for control fish. A linear regression analysis showed that well fed fish had less
31 of a stress response compared to less well fed individuals. Further, a discriminant
32 function analyses indicated that the suite of biomarkers used were successful in
33 indicating different stress responses according to angling duration. Angling and
34 handling times along with nutritional status were likely influential factors in the range
35 of glucose, cortisol and especially lactate levels in this study. These data suggest that

C&R causes physiological stress to tigerfish, but could nonetheless be a valuable fisheries management tool, ensuring the sustainability of fish populations.

Keywords: cortisol, glucose, lactate, plasma biomarkers, univariate statistics, discriminant function analysis

INTRODUCTION

The tigerfish, *Hydrocynus vittatus* (Castelnau 1861) is one of Africa's primary predatory freshwater fish (Winemiller and Kelso-Winemiller 1994; Skelton, 2001; Økland et al. 2005) and is considered to be Africa's most sought after freshwater gamefish (Smit et al. 2009). Tigerfish put up a strong fight when angled, with jumping displays similar to that of dorado, *Coryphaena hippurus* (Linnaeus 1758), and sailfish, *Istiophorus albicans* (Latreille 1804). One of the most popular angling destinations for tigerfish is the panhandle region of the Okavango Delta, which features deeper faster flowing waters than in the rest of the Delta (Smit et al. 2009). Many recreational anglers frequent the numerous tourist camps and lodges in the area particularly during the annual catfish migrations during late August and early September (Smit et al. 2009).

Recreational angling has developed into a valuable activity, which generates considerable income for both local and national economies (Cooke and Suski 2005). In recent years there has been a marked increase in fishing lodges in the Okavango Delta area and as such the numbers of boats and recreational fishermen have also increased, hence escalating fishing pressure in the area (Pers. Obs). Most captured fish are released shortly afterwards, either voluntarily or because of permit requirements. In Botswana a recreational angling licence is a requirement for anglers visiting the area. The license stipulates that only 5 fish, regardless of species, may be kept daily. The remainder (including all juvenile and undersized fish) must be released. Fish species mostly kept by recreational anglers are bream species such as the nembwe *Serranochromis robustus* (Günther 1864), three-spot tilapia *Oreochromis andersonii* (Castelnau 1861), and thinface large mouth, *Serranochromis angusticeps* (Boulenger 1907), as these are considered the best eating fish in the region (Van Dyk et al. 2009). *Hydrocynus vittatus* is not popular as an eating fish and generally not kept by recreational fisherman; however it does form the basis of the catch-and-release (C&R) fishery in the Okavango Delta. The only *H. vittatus* specimens generally kept are trophy fish, normally mounted for display. This licensing system does not provide the means for fisheries managers to determine the fishing pressure placed on *H.*

71 *vittatus*, due to tigerfish mostly being released after capture and as such no indication
72 or data is available as to how many *H. vittatus* are actually being caught and landed.

73 Information pertaining to the effect of angling on Africa's premier game fishes
74 is scanty (Smit et al. 2009). Most available information concerns popular European
75 and North American species (Brobbel et al. 1996; Cooke et al. 2002; Dubois and
76 Dubelzig 2004; Mclean et al. 2016), namely, largemouth bass, cutthroat trout and
77 various salmon species, respectively. The only previous work that has investigated the
78 physiological stress induced from C&R in any African freshwater system was firstly, a
79 scoping study by Smit et al. (2009) on *H. vittatus*, but this work only examined blood
80 lactate concentrations, using rapid assessment techniques (hand held lactate meter).
81 In the second and to our knowledge the only other paper, Smit et al (2016) assessed
82 plasma levels of lactate, glucose and cortisol of the Vaal-Orange smallmouth
83 yellowfish (*Labeobarbus aeneus*) following capture by fly-fishing.

84 The aims of this study were (1) to examine the response of tigerfish to C&R
85 angling using blood plasma levels of glucose, cortisol and lactate as physiological
86 stress biomarkers and compare the data to those of Smit *et al.* (2009, 2016) and the
87 other relevant European and North American species, (2) identify whether the chosen
88 set of biomarkers are suitable in assessing the stress response of angled tigerfish.

90 MATERIALS AND METHODS

91 **Study area**

92 The Okavango Delta is a subtropical floodplain, situated in the northern end of the
93 Kalahari Desert in Botswana (Figure 1). During the seasonal floods, the delta
94 fluctuates between 12000 – 15000 km², and during the dry season between 6000 –
95 8000 km² (Merron and Mann 1995). The study area (Figure 1) was situated in the
96 panhandle region of the Okavango, just north of the Delta itself, close to the town of
97 Shakawe and is characterised by slow flowing, meandering water.

99 **Sampling**

100 The University of Johannesburg's Institutional Research Ethics Committee ratified all
101 experimental procedures prior to data collection. *Hydrocynus vittatus* (n=63) were
102 captured during the annual catfish feeding migrations of September (spring) 2008 by
103 means of standard recreational angling. Fisherman were encouraged to fish as normal
104 (fighting fish as preferred). The primary gear setup used during this study was a seven
105 foot rod rigged with spinning reels fitted with 4 kg braided line and artificial lures
106 (crankbaits). Anglers that participated in this study consisted of a group of researchers

with varying degrees of fishing experience. The more experienced group of fisherman had been fishing for *H. vittatus* in this area for more than 3 years (range 3 to 10 years), whereas the novice anglers had < 2 years fishing experience. For each fish caught the times to land fish and handling procedure (hook removal) were recorded. Landing time refers to the time from when the fish was hooked until it was landed (caught in the net); the handling time refers to the time from when the caught fish is netted, the hook removed and the fish placed under anaesthesia. Landing and handling times for each fish were combined and referred to as the total angling time.

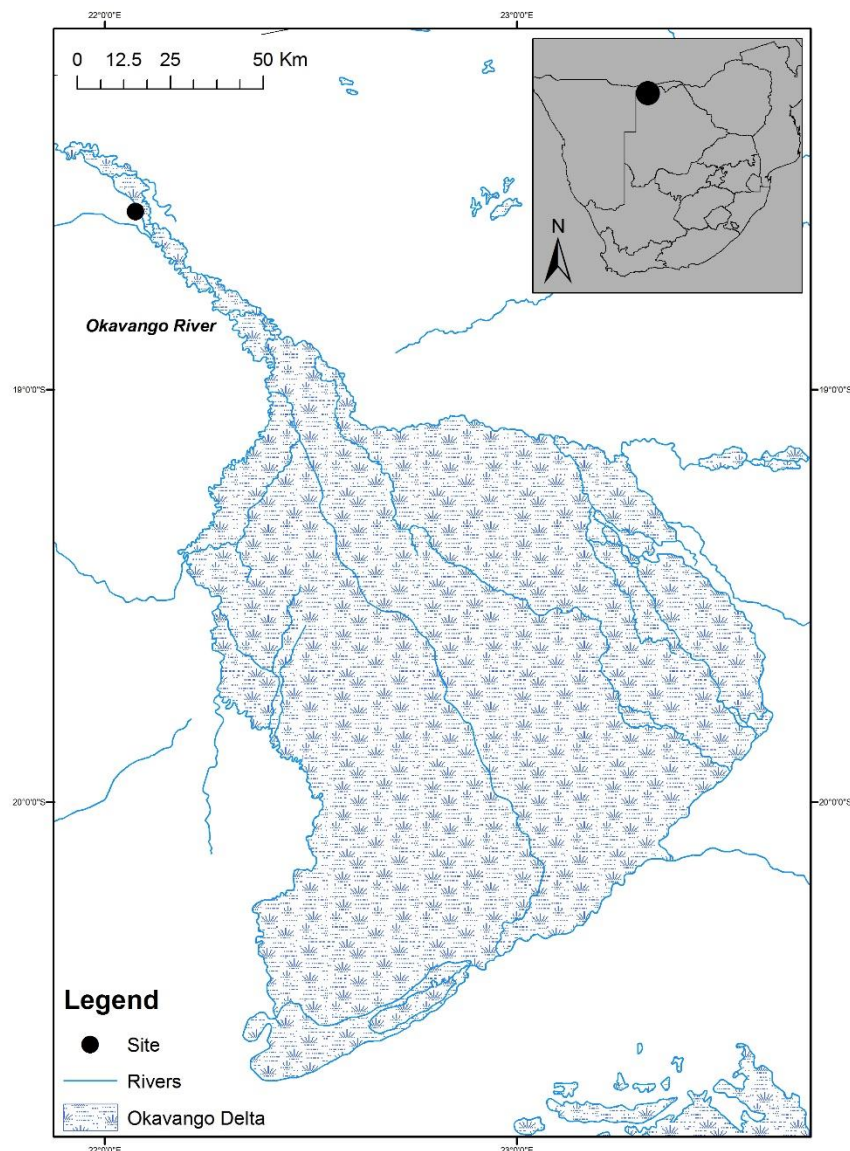


Figure 1: Map of the Okavango Delta in Botswana, the marker indicates the sampled area in the north of the Delta (panhandle).

Anaesthesia and Sampling procedure

Following capture, all fish were anaesthetised for 2 min in a 96 L container containing 50 L of fresh river water with a 32 mg L⁻¹ concentration of clove oil solution [1:9 ratio of clove oil mixed with ethanol] (Anderson et al. 1997; Meka and McCormick 2005). Anaesthetised fish were removed from the container and 2 mL of blood was drawn from the caudal vein (time < 30 s) using sterile 1 mL syringes and 21-gauge needles. Blood was immediately transferred to 4 mL heparinised vacutainers and kept cool until centrifugation to separate the plasma supernatant. Plasma was stored in liquid nitrogen in the field and then stored at -80 °C on return to the laboratory. In addition to the timings (landing and handling times), general fish characteristics (mass and various length measurements: standard length [SL], fork length [FL] and total length [TL]) were recorded after blood had been drawn. On completion of sample and data collection, each fish was revived in flowing river water and released. The general fish characteristics of mass and length were used to calculate a condition factor [K] for each fish following Carlander (1969):

$$K = \frac{100000W}{L^3}$$

where: W = the weight of the fish in grams;

L = the standard length of the fish in millimeters

The condition factor is a reflection of the fish's relative robustness or its degree of well-being.

Sample Analysis

Plasma glucose and lactate were determined using Roche/Hitachi kits (Mannheim, Germany; Model no. 11448668 216 [CV=1.8%] and model no. 11822837 190 [CV=1.0%] respectively). The volumes required for the glucose and lactate analysis were adapted so that the reactions could take place in 300 µl microplate wells. The glucose and lactate test kits used consist of glucose oxidase and lactate oxidase, respectively. The oxidation of glucose, forms gluconate and hydrogen peroxide and the oxidation of lactate forms pyruvate and hydrogen peroxide. After these reactions, peroxidase catalyses a reaction with the hydrogen peroxide to form a colour and the intensity of this colour is then measured. These specific lab based assays have been used extensively in fish based analyses (Venn Beecham et al. 2006). Plasma cortisol was determined through ELISA, (Model no. 402710 [CV=9%], Neogen Corporation,

Lexington, Kentucky USA). The ELISA used consists of rabbit antibody, horseradish peroxidase conjugate and 3,3',5,5'-tetramethylbenzidine as substrate. This specific assay was used as its validity for use in fish was established by Barry et al (1993). More recently Velasco-Santamaria and Cruz-Casallas (2007) also showed that, cortisol ELISA's developed for human plasma analyses is an efficient and accurate method for cortisol determination in fish. Plasma glucose, cortisol and lactate assays were run on a Biotek microplate reader at wavelengths of 540 nm (glucose) and 630 nm (cortisol and lactate). Concentrations were calculated from the absorbances for each assay. The most influential factors contributing to changes in the physiological response were considered to be both landing and handling time, because of the exhaustive nature of hooking, landing and unhooking processes (Booth et al. 1995; Meka and McCormick 2005). As such, physiological changes caused by landing and handling times were analysed separately as well as in combination (total time).

Reference group

A reference group consisted of 10 randomly chosen fish. Following the initial blood drawing, fish were revived in fresh river water and kept in an aerated 96L insulated container filled with fresh river water. These control fish were transported to the field laboratory within 60 min where they were transferred to an aquarium containing 12 000 L of fresh river water. Air pumps were used to continually aerate the water. Approximately 20% of the aquarium water was replaced daily to provide fish with fresh river water (Smit et al. 2009). Fish were left undisturbed for a 72 h period (Gustaveson et al. 1991; Smit et al. 2009; 2016), to allow the physiological stress response from initial capture to subside and emulate the condition of free swimming unstressed fish. After 72 h clove oil was added to the water to anaesthetise (2 min) the fish and a further blood sample was taken and analysed as previously described. Samples from this group of fish were used as a reference to examine the differences with values attained from captured fish.

Data analysis

All data were analysed using SPSS for windows v. 18 (PASW Statistics, IBM, USA). All descriptive data are reported as mean \pm standard deviation (SD). Pearson's Correlation Coefficient was used to examine the influence of the various fish characteristics on the different angling variables and biomarkers. Differences between post capture and following 72 h in an aquarium were analysed for plasma glucose, cortisol and lactate using a paired samples t-test. In line with previous research, fish

were grouped by landing times and total angling times, which were divided into minute intervals (< 1 min, 1–2 min, 2–3 min, etc). A one-way ANOVA was used to examine the plasma cortisol, plasma glucose, and plasma lactate responses resulting from the different angling times. Differences between groups were determined with the aid of an LSD *post-hoc* test. Significance levels were set *a priori* at $\alpha = 0.05$. A discriminant function analysis (DFA) was performed for sampled fish to determine whether the biomarkers could be used to reclassify individual samples into their respective landing time groupings. The various groups assessed are listed above apart from the >3 min group which was excluded as it had too few cases. *A posteriori* tests using Fisher's function coefficients were used to examine the probability of membership of individual samples to each of the abovementioned groups. Canonical variates for each sample were also produced during the analysis, based on the eigen structure of the multivariate matrix, of which the first two variates were plotted to determine patterns in the data. The reclassification success from the DFA was used to estimate variability in the physiological stress of angling duration between groups.

RESULTS

Mean total length (TL) and body mass \pm SD and range of the caught fish were 586 ± 74 mm (405 – 765 mm) and 1.96 ± 0.91 kg (0.48 – 5.24 kg). Mean total angling time was 1 min 54 s \pm 54 s (31 s – 4 min 57 s) with mean landing and handling times \pm SD and range of 1 min \pm 39 s (16 s – 4 min 34 s) and 51 s \pm 29 s (10 s – 2 min 14 s), respectively. A significant positive correlation was found between body mass and the various angling times as well as fish condition and the various angling times (Table 1). Following 72 h in an aquarium, the mean \pm SD and range for plasma glucose, plasma cortisol and plasma lactate concentrations in the control fish was 4.1 ± 0.97 mMol·L⁻¹ (3 – 5.27 mMol L⁻¹); 36.67 ± 25.74 ng mL⁻¹ (12.09 – 80.16 ng mL⁻¹); and 3.41 ± 1.33 mMol L⁻¹ (1.34 – 5.03 mMol L⁻¹), respectively. Plasma lactate concentrations in these individuals were significantly higher following hook and line capture, 7.42 ± 2.96 mMol L⁻¹ (3.43 – 11.75 mMol L⁻¹) than the concentrations found in the same fish following 72 h in an aquarium ($t = 2.41$, $df = 9$, $p = 0.006$); however, both plasma glucose ($t = 1.45$, $df = 10$, $p = 0.044$) and plasma cortisol ($t = 2.72$, $df = 9$, $p = 0.011$) levels from control fish following 72 h in an aquarium were significantly higher than in the same individuals post capture.

Landing time and angling duration

Plasma lactate concentrations were found to be significantly different when grouped by land time and total time ($F = 6.8$, $df = 67$, $p = 0.001$; $F = 5.2$, $df = 67$, $p = 0.001$) (Figure 2 panel A, B). The LSD post-hoc analyses showed differences between plasma lactate concentrations in the control group and all the other landing and total time groups: < 1 min ($p < 0.05$); $1 - 2$ min ($p < 0.05$); $2 - 3$ min ($p < 0.05$); and > 3 min ($p < 0.05$). Further one way ANOVAs revealed that plasma cortisol (Figure 2 panel C, D) ($F = 1.23$, $df = 66$, $p = 0.31$; $F = 0.91$, $df = 66$, $p = 0.47$) and plasma glucose (Figure 2 panel E, F) ($F = 0.56$, $df = 69$, $p = 0.69$; $F = 0.82$, $df = 69$, $p = 0.52$) concentrations were not significantly different between groups when calculated using both land time and total time. Although differences were found in the lactate response between groups vs. the control group, there was no correlation between the angling durations and any of the plasma biomarker responses (Table 1). Glucose showed a significant, but nonetheless weak correlation with fish mass and a significant negative correlation to plasma lactate levels. Interestingly plasma lactate levels were negatively correlated to fish condition (Table 1).

Angling stress group assessments

The plasma biomarker data were able to reclassify 52.7% of the samples into their predefined angling time categories (Table 2). Reclassification errors consisted of some overlap in stress responses between the different angling times, except for the control group which was distinct from any of the other groups. From the DFA (Figure 3) it is clear that the lactate response had the greatest effect upon the grouping of an individual into a specific group most notably on function 1, with the glucose and cortisol having some effect on the second function.

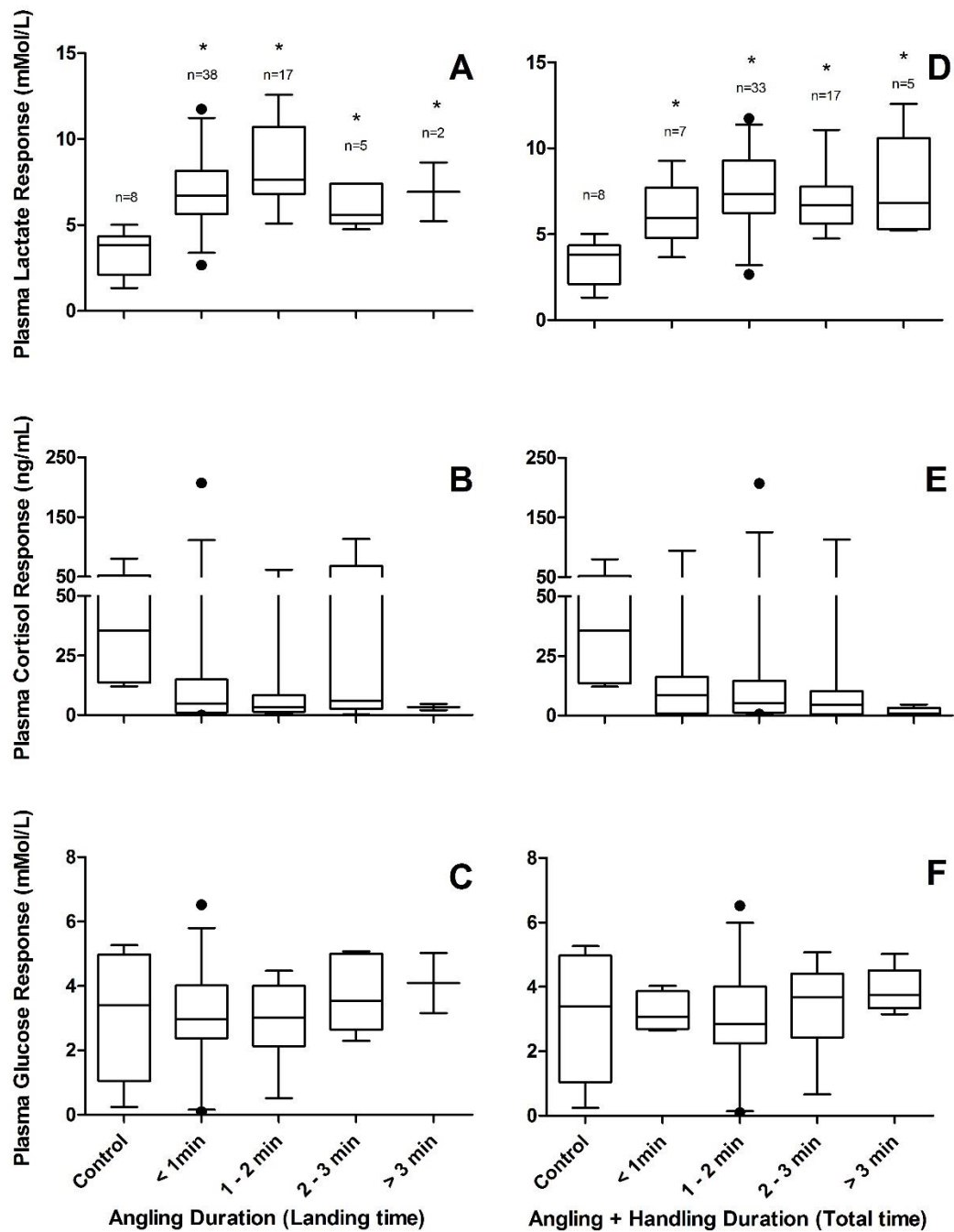


Figure 2: Plasma lactate (A and B), cortisol (C and D) and glucose (E and F) responses of control and angled *Hydrocynus vittatus*, for both angling and total times, respectively. Values are presented as box and whisker plots (5 – 95%) and filled circles indicate outliers. * denotes that blood lactate concentrations were significantly greater than the control group ($P < 0.05$).

Table 1: Pearsons correlation coefficients for plasma angling stress parameters in *Hydrocynus vittatus* and the various angling times and specimen data.

Correlations								
	Mass	Land	Hand	Total	Condition	Glucose	Lactate	Cortisol
Mass	1							
Land	.639**	1						
Hand	-.147	-.132	1					
Total	.443**	.790**	.503**	1				
Condition	.565**	.330*	.005	.434**	1			
Glucose	.286*	.231	.082	.208	.092	1		
Lactate	-.262	-.093	-.042	-.042	-.313*	-.375**	1	
Cortisol	-.004	.001	-.130	-.101	.006	-.176	.118	1
**. Correlation is significant at the 0.01 level (2-tailed).								
*. Correlation is significant at the 0.05 level (2-tailed).								

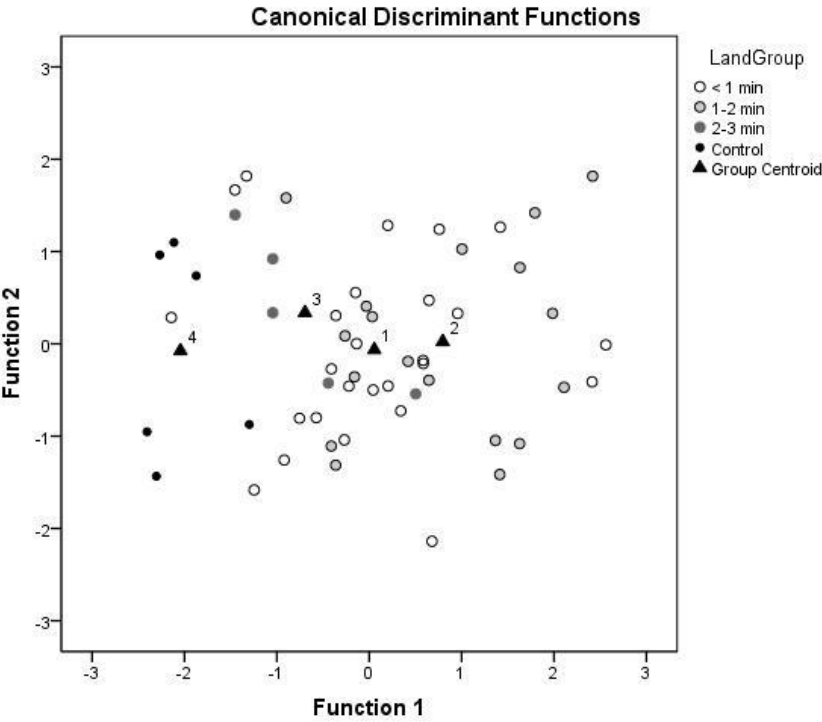


Figure 3: Canonical variates derived from a discriminant function analysis (DFA), using plasma biomarker data from individual samples of *Hydrocynus vittatus* angled for various time intervals. Function 1 and 2 refer to the first two canonical functions of a multivariate data set.

Table 2: Classification summary of the various time intervals from discriminant function analyses (DFA) of plasma biomarkers in *Hydrocynus vittatus* from the Okavango Delta.

Classification Results ^a								
		Land Group		Predicted Group Membership				Total
				< 1 min	1 – 2 min	2 - 3 min	Control	
	%		< 1 min	38.5	34.6	19.2	7.7	100.0
			1 – 2 min	38.9	55.6	5.6	.0	100.0
			2 - 3 min	20.0	20.0	60.0	.0	100.0
			Control	.0	.0	.0	100.0	100.0
a. 52. 7 % of original grouped cases correctly classified.								

DISCUSSION

The aims of this paper were two-fold where firstly the blood plasma biomarkers of lactate, cortisol and glucose were used to examine the physiological response of tigerfish to C&R angling and secondly to assess whether the chosen biomarkers are suitable to examine this physiological response. The key findings included that lactate was the most suitable of the three selected variables in examining the physiological response and interestingly that condition factor played a role in this response. This study adds to the existing body of knowledge of C&R angling and even more so in terms of Africa's prominent angling species for which there is very limited data.

Effect of size (body mass) and fish condition

When compared to the work of Smit et al. (2009), a similar number of fish were angled (63 vs. 66) using similar angling gear. Fish captured in this study were slightly larger and heavier than those of Smit et al. (2009), and thus had longer total and landing, angling durations. As the body mass of the angled tigerfish increased, so did the angling duration. This has been shown for many species, namely; Atlantic salmon (Thorstad et al. 2003), rainbow trout (Meka and McCormick 2005) yellowfish (Smit et al. 2016) and tigerfish in the study of Smit et al. (2009). Although the sample size was similar to those previously angled by Smit et al. (2009), these authors showed a stronger positive correlation between body mass with angling duration. This discrepancy may be attributed to the fact that the current study made use of more anglers (n=8), and each angler has his own way of fighting the fish. In addition, some of the anglers were novices and thus may have fought the fish for longer periods of time. The levels of cortisol and lactate showed no correlation to body mass, whereas glucose levels did show a positive correlation to body mass. Smit et al. (2009) also found that lactate concentrations of control fish data were independent of body mass

and concluded that the metabolic stress caused by angling stress was the chief cause of elevated blood lactate levels (Meka and McCormick 2005). Similarly, increased plasma cortisol and lactate levels in this study are attributed to the physiological stresses of angling. The negative correlation between plasma lactate and glucose, was indicative of the work fish were doing under the physical stresses of angling. Where the glucose usage is linked to both the aerobic and anaerobic glycolytic pathways for energy production during angling. The lactate levels are the by-product of the anaerobic metabolism which suggests that there is a great reliance on anaerobic metabolism whilst fish are fighting during angling. Interestingly a negative correlation was found between the plasma lactate response and the fish's condition factor. This does not necessarily imply that individuals, that had a lesser angling stress response (in terms of plasma lactate), were in better condition as a result of the physical fitness of the individual fish. Instead it might indicate that fish that have a higher mass to length ratio, had a lower lactate response than those individuals which were considered to not be as well fed in terms of the condition factor. This has been shown for largemouth bass and is attributed to the availability of energy stores in well fed fish, see discussion in Gingerich et al. (2010).

Effect of the angling period

The blood parameters tested from angled tigerfish exhibit increased cortisol (in some individuals) and increased plasma lactate concentrations with increased angling time as well as total angling duration, while glucose levels did not vary significantly. The plasma lactate response of *H. vittatus* to angling duration was significantly greater than controls at every time interval, indicating a large degree of metabolic stress, even after shorter angling durations of less than 1 min, which supports previous work (Smit et al. 2009; 2016). Tigerfish showed an immediate response with significantly higher blood lactate in fish angled for 1 min and throughout the increasing time intervals when compared to controls. Smit et al. (2009) showed elevated levels in less than 1 min (3.2 mMol.L⁻¹) after hooking when compared to control fish (1.6 mMol.L⁻¹), this is similar to the current study where control levels (3.4 mMol.L⁻¹) of plasma lactate were around half of that of fish that were angled for less than 1 min (6.9 mMol.L⁻¹)

According to Thorstad et al. (2003), handling induces primary, secondary and tertiary stress responses. Handling duration has been shown to negatively affect angled fish even further (Meka and McCormick 2005), by impairing ventilation and resulting in further muscular exertion (Arlinghaus and Hallerman 2007). Mortality has also been shown to increase with air exposure (Arlinghaus and Hallerman 2007); while out of water the gill lamellae collapse and inhibit gas exchange, further affecting acid-

base balance that conceptually leads to further physiological disturbances and results in longer recovery times (Arlinghaus and Hallerman 2007). In a study on pikeperch Arlinghaus and Hallerman (2007) found that fish with no air exposure had the lowest mortality rates, whilst fish that were exposed to air showed no significant difference between shorter and longer duration air exposure times – thus even short durations of air exposure can result in increased mortality rates. The potential effects of angling stress at a population level from both the angling duration and the handling time are well discussed by Smit et al. (2009), which is especially poignant when one considers the largest fish are mature females (males do not tend to grow larger than 2 kg (Gerber et al. 2009)) and hence take longer to angle and therefore undergo the greatest physiological stress.

The 10 reference fish were kept successfully for 72 h and their plasma lactate levels were significantly lower than the post-capture levels (3.4 mMol.L^{-1} vs 7.5 mMol.L^{-1} , respectively). Control fish plasma lactate levels in this study are higher than the results of Smit et al. (2009), but it is important to note that the techniques used for determining blood lactate levels differed from that used in the present study. The mean cortisol value of the angled *H. vittatus* was low and showed no increase with angling time. Post capture values of cortisol were significantly different from the control fish data, in that control fish had higher cortisol levels; this is most probably due to the stress of being confined to a smaller environment and not because levels did not recover to reflect those of free-swimming fish. Consequently, we propose that the cortisol values found during this study are also reflective of free-swimming “unstressed” fish. A few individuals ($n=13$) did show elevated cortisol concentrations of $15 - 207 \text{ ng.mL}^{-1}$. This might indicate that for fish with non-elevated levels, the drawing of blood may have been done before the cortisol response could be measured. The lack of an increase in the glucose concentrations provided further support to the notion that the cortisol concentrations measured in this study are likely a reflection of resting values, as greater circulating cortisol concentrations tend to result in higher glucose concentrations (Barton 2002; Iwama et al. 2004). Based on the data from the reference fish we are confident that lactate and glucose levels in the reference group returned to concentrations that are analogous to the resting/free swimming levels of the natural population.

The discriminant function analyses proved a useful tool to determine whether the plasma biomarker responses were distinct for reference fish and all other angling time groups. The plasma lactate response proved to be the most useful biomarker to discriminate between groups and can be attributed to its rapid formation in the presence of physical work while being angled. The plasma glucose and cortisol

biomarkers also proved useful in the reference group separating from the angled fish. However, these aforementioned biomarkers were not successful in discriminating between the different angling times. The DFA also indicated overlap between the different angling time fish. This shows that the plasma biomarkers used in this study are suitably sensitive markers in the analyses of angling stress, although some overlap in the stress response between the defined angling period groups is indicated. This overlap is caused by the fish close to the boundaries of the predefined groups.

CONCLUSIONS

The results obtained in this study directly apply to *H. vittatus* in the panhandle area of the Okavango Delta, Botswana, but might be extrapolated to other popular catch-and-release populations throughout Southern Africa. Angling and handling times along with nutritional status were likely influential factors in the range of glucose, cortisol and especially lactate levels in this study. When the results of this study were compared to that of other freshwater species such as rainbow trout, largemouth bass, smallmouth yellowfish and other work on tigerfish, it was clear that the physiological response to angling is species specific, therefore it will be important that future angling induced stress research should focus on all the different targeted freshwater game fish species in Africa.

Acknowledgements

These results were obtained thanks to the support of AIRES-Sud, a programme of the French Ministry of Foreign and European Affairs implemented by the Institut de recherche pour le Développement (IRD-DSF). We thank the University of the Free State for the use of their Leseding research camp and Dr Wynand Malherbe for the map of the study area. This is contribution number 192 from the NWU-Water Research Group.

References

- Anderson WG, McKinley RS, Colavecchia M. 1997. The use of clove oil as an anaesthetic for rainbow trout and its effects on swimming performance. *North American Journal of Fisheries Management* 17: 301–307.
- Arlinghaus R, Hallerman J. 2007. Effects of air exposure on mortality and growth of undersized pikeperch, *Sander lucioperca*, at low water temperatures with implications for catch-and-release fishing. *Fisheries Management and Ecology* 14: 155–160.

412 Barry TP, Lapp AF, Kayes TB, Malison JA. 1993. Validation of a microtitre plate
413 ELISA for measuring cortisol in fish and comparison of stress responses of rainbow
414 trout (*Oncorhynchus mykiss*) and lake trout (*Salvelinus namaycush*). *Aquaculture*
415 117: 351–363.

416 Barton BA. 2002. Stress in fishes: A diversity of responses with particular reference to
417 changes in circulating corticosteroids. *Integrated and Comparative Biology* 42: 517–
418 525.

419 Booth RK, Kieffer JD, Davidson K, Bielak AT, Tufts BL. 1995. Effects of late-season
420 catch and release angling on anaerobic metabolism, acid-base status, survival and
421 gamete viability in wild Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries*
422 *and Aquatic Sciences* 52: 283–290.

423 Brobbel MA, Wilkie MP, Davidson K, Kieffer JD, Bielak AT, Tufts BL. 1996.
424 Physiological effects of catch and release angling in Atlantic salmon (*Salmo salar*) at
425 different stages of freshwater migration. *Canadian Journal of Fisheries and Aquatic*
426 *Sciences* 53: 2036–2043.

427 Carlander KD. 1969. Handbook of freshwater fishery biology. Iowa State University
428 Press: Iowa.

429 Cooke SJ, Suski CD. 2005. Do we need species specific guidelines for catch-and-
430 release recreational angling to effectively conserve diverse fishery resources?
431 *Biodiversity and Conservation* 14: 1195–1209.

432 Cooke SJ, Schreer JF, Wahl DH, Philipp DP. 2002. Physiological impacts of catch-
433 and-release angling practices on largemouth bass and smallmouth bass. In *Black*
434 *Bass: Ecology, Conservation, and Management* (Ridgway, M. & Philipp, D., eds).
435 Bethesda, MD: American Fisheries Society. pp. 489–512.

436 DuBois RB, Dubielzig RR. 2004. Effect of hook type on mortality, trauma and capture
437 efficiency of wild stream trout caught by angling with spinners. *North American*
438 *Journal of Fisheries and Management* 24: 609–616.

439 Gerber R, Smit NJ, Pieterse GM, Durholtz D. 2009. Age estimation, growth rate and
440 size at sexual maturity of tigerfish *Hydrocynus vittatus* from the Okavango Delta,
441 Botswana. *African Journal of Aquatic Science* 34: 239–247.

442 Gingerich AJ, Philipp DP, Suski CD. 2010. Effects of nutritional status on metabolic
443 rate, exercise and recovery in a freshwater fish. *Journal of Comparative Physiology*
444 180: 371–384.

445 Gustaveson AW, Wydoski RS, Wedemeyer GA. 1991. Physiological response of
 446 largemouth bass to angling stress. *Transactions of the American Fisheries Society*
 447 120: 629–636.

448 Iwama GK, Afonso LOB, Vijayan MM. 2004. Stress in fish. Aquanet workshop on fish
 449 welfare, Campbell River, B.C. Canada. 27 September 2004.

450 Mclean MF, Hanson KC, Cooke S, Hinch SG, Patterson DA, Nettles TL, Litvak MK,
 451 Crossin GT. 2016. Physiological stress response, reflex impairment and delayed
 452 mortality of white sturgeon *Acipenser transmontanus* exposed to simulated fisheries
 453 stressors. *Conservation Physiology* DOI: 10.1093/conphys/cow031

454 Meka JM, McCormick SD. 2005. Physiological response of wild rainbow trout to
 455 angling: impact of angling duration, fish size, body condition and temperature.
 456 *Fisheries Research* 72: 311–322.

457 Merron GS, Mann BQ. 1995. The reproductive and feeding biology of *Schilbe*
 458 *intermedius* Rüppell in the Okavango Delta, Botswana. *Hydrobiologia* 308: 121–129.

459 Økland F, Thorstad EB, Hay CJ, Næsje TF, Chanda B. 2005. Patterns of movement
 460 and habitat use by tigerfish (*Hydrocynus vittatus*) in the Upper Zambezi River
 461 (Namibia). *Ecology of Freshwater Fish* 14: 79–86.

462 Smit NJ, Howatson G, Greenfield R. 2009. Blood lactate levels as biomarker for
 463 angling induced stress in the tigerfish *Hydrocynus vittatus* from the Okavango Delta,
 464 Botswana. *African Journal of Aquatic Sciences* 34: 255–259.

465 Smit NJ, Gerber R, Greenfield R, Howatson G. 2016. Physiological response of one
 466 of South Africa's premier freshwater sport angling species, the Vaal-Orange
 467 Smallmouth Yellowfish, *Labeobarbus aeneus*, to catch-and-release angling. *African*
 468 *Zoology* 51: 61 – 67.

469 Skelton P. 2001. A Complete Guide to Freshwater Fishes of Southern Africa. Struik
 470 Publishers, Cape Town.

471 Thorstad EB, Næsje TF, Fiske P, Finstad B. 2003. Effects of hook and release on
 472 Atlantic salmon in the River Alta, northern Norway. *Fisheries Research* 60: 293–307.

473 Van Dyk C, Marchand M, Smit NJ, Pieterse GM. 2009. A histology-based fish health
 474 assessment of four commercially and ecologically important species from the
 475 Okavango Delta panhandle, Botswana. *African Journal of Aquatic Science* 34: 273–
 476 282.

- 477 Velasco-Santamaria Y, Cruz-Cansallas P. 2007. Methodology for determination of
478 plasma cortisol in fish using competitive Enzyme-Linked Immunosorbent Assay
479 (ELISA). *Revista MVZ Córdoba* 12: 869–877.
- 480 Venn Beecham R, Small BC, Minchew BC. 2006. Using portable lactate and glucose
481 meters for catfish research: acceptable alternatives to established laboratory
482 methods? *North American Journal of Aquaculture* 49: 291–295.
- 483 Winemiller KO, Kelso-Winemiller LC. 1994. Comparative ecology of the African pike,
484 *Hepsetus odoe*, and tigerfish, *Hydrocynus forskahlii*, in the Zambezi River floodplain.
485 *Journal of Fish Biology* 45: 211–225.